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An Unusual Presentation of McCune-Albright Syndrome Confirmed by an Activating Mutation of the G_s α-Subunit from a Bone Lesion*

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ABSTRACT

The McCune-Albright syndrome (MAS) is characterized clinically by polyostotic fibrous dysplasia, café-au-lait skin lesions, sexual precocity, and various other endocrinopathies. Recent investigations suggest an etiological role for embryonic somatic missense mutations that predict the substitution of a His or Cys for Arg at amino acid 201 of the G_s α-subunit ($G_s\alpha$). Identification of these mutations in affected tissues is a sensitive assay that may help define a more complete clinical spectrum of the MAS. We investigated a woman who developed fibrous dysplasia 24 yr after premature menstruation. To determine if this was an unusual MAS variant, DNA and RNA were analyzed from affected and unaffected tissues.

From samples of affected rib and normal rib DNA was extracted, amplified by polymerase chain reaction, subcloned, and sequenced. RNA was extracted from affected bone, reverse transcribed, amplified by polymerase chain reaction, subcloned, and sequenced. DNA sequence predicting a His for Arg substitution at $G_s\alpha$ amino acid 201 was found in 47% of the recombinant plasmids from DNA of affected bone and 17% of the plasmids from DNA of unaffected bone; a significant ($P < 0.05$) difference in frequency. The His^{201} substitution was found in 42% of the recombinant plasmids from RNA of affected bone. We conclude that this clinical variant is qualitatively indistinguishable from presentations of the complete MAS. (*J Clin Endocrinol Metab* **78:** 803–806, 1994)

THE MCCUNE-ALBRIGHT syndrome (MAS) is an uncommon clinical disorder characterized by café-au-lait skin lesions, polyostotic fibrous dysplasia, sexual precocity, and other endocrinopathies (1–5). These protean manifestations are probably caused by embryonic somatic mutations leading to the substitution of His or Cys for Arg at amino acid 201 of the α-subunit of the signal transduction protein G_s ($G_s\alpha$) (6, 7). This mutation inhibits the GTPase activity of $G_s\alpha$, and adenylate cyclase is constitutively activated. The clinical characteristics are postulated to be caused by autonomous signaling in tissues, such as endocrine organs, which express $G_s\alpha$-linked signaling mechanisms. Identification of $G_s\alpha$ mutations may help to define a more complete clinical spectrum of the MAS.

In this study we investigate the $G_s\alpha$ sequence in normal bone and the lesion of fibrous dysplasia in a subject with a potential variant of the MAS. Fibrous dysplasia developed 25 yr after transient premature menstruation and sexual precocity.

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Case Report

A 27-yr-old woman developed fibrous dysplasia 24 yr after transient premature menstruation and sexual precocity. She had presented at age 3 yr with vaginal bleeding. Breast development was noted at age 4 yr, and a second episode of vaginal bleeding was observed at age 5 7/12 yr. She had no café-au-lait skin lesions, and two sets of radiographs of the chest, pelvis, skull, and long bones were normal. She was treated for 24 months with medroxyprogesterone acetate, and precocity resolved. She progressed normally through puberty, starting at age 10 yr, and had normal menses, starting at age 12 yr. At age 25 yr, she delivered a normal daughter. A chest x-ray at age 27 yr after minor trauma identified a single lesion of fibrous dysplasia. Her height was 165 cm, weight was 86 kg, blood pressure was 120/80 mm Hg, and pulse was 78 bpm. No café-au-lait skin lesions were found. The visual fields were full to confrontation, and the thyroid gland was of normal size without nodules. Radiographs of the ribs and long bones showed a single diffuse expansile lesion in the left seventh rib. Serum concentrations of electrolytes, including phosphate and calcium, were normal. Circulating concentrations of T_4, T_3, TSH, cortisol, ACTH, PRL, GH, and insulin-like growth factor-I were normal.

Materials and Methods

The subjects gave informed consent for this study, which was approved by the Institutional Review Board of the University of Connecticut Health Center.

Hormone assays

Serum cortisol concentration was measured by high pressure liquid chromatography (8). Plasma ACTH and serum TSH concentrations were measured by immunoradiometric assay at Nichols Institute (San Juan Capistrano, CA). Other serum hormone concentrations were determined by RIA.

DNA and RNA extraction

DNA was extracted from frozen pulverized bone and from mononuclear leukocytes (MNL) by the method of Gross-Bellard *et al.* (9). MNL were prepared by density gradient centrifugation with Ficoll-Hypaque (10). RNA was extracted from frozen pulverized bone using the guanidinium isothiocyanate method (11).

DNA amplification, subcloning, and sequencing

Figure 1 shows the strategy for amplifying, subcloning, and sequencing the regions of interest of the $G_s\alpha$ gene (Fig. 1A) and $G_s\alpha$ cDNA (Fig. 1B), and Table 1 shows the primers. Polymerase chain reaction (PCR) primers 1 and 2 were described by Lyons *et al.* (12). The numbering of $G_s\alpha$ cDNA nucleotides and amino acids is according to Kozasa *et al.* (13). All PCR reactions were carried out with 12.5 pmol of each primer in a final volume of 100 μL containing 50 mmol/L KCl, 10 mmol/L Tris (pH 8.3), 1.5 mmol/L $MgCl_2$, 0.01% gelatin, 200 μmol/L deoxy-NTPs, and 2.5 U *Taq* polymerase (14).

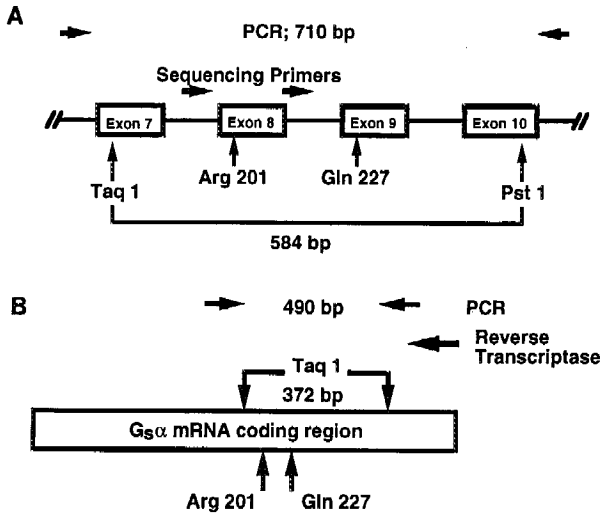

FIG. 1. Strategy for amplifying and subcloning selected regions of the $G_s\alpha$ gene (A) and Gs_α cDNA (B). The approximate positions of Arg^{201} and Gln^{227} within the gene and cDNA are indicated. The primers for sequencing, PCR amplification, and reverse transcription of RNA are indicated by the *horizontal arrows*, and the sequences of these primers are given in Table 1. The restriction enzyme sites used for subcloning are shown.

Amplification of DNA from bone (PCR primers 1 and 2) yielded a 710-basepair (bp) fragment that was cleaved with *Taq*I and *Pst*I and subcloned into Bluescript (Stratagene, La Jolla, CA). The mRNA from affected bone was reverse transcribed into cDNA. Total RNA (5 μg) was incubated for 60 min at 37 C with 2.5 μg reverse transcriptase primer in 50 μL buffer containing 50 mmol/L Tris (pH 8.3), 75 mmol/L KCl, 3 mmol/L $MgCl_2$, 10 mmol/L dithiothreitol, 500 μmol/L deoxy-NTPs, and 250 U Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD). The region of interest was PCR amplified from 4 μL of the reverse transcriptase mixture using PCR primers 4 and 5, cleaved with *Taq*I, and subcloned into Bluescript. Recombinant plasmids were sequenced by the dideoxy method (15).

Statistical significance was determined by χ^2 analysis.

Restriction enzyme assay

The A for G substitution in the $G_s\alpha$ gene predicts a new *Nla*3 restriction enzyme site. DNA (150 ng) from the indicated sources was PCR amplified by PCR primers 1 and 3, yielding a 354-bp product. The PCR-amplified DNA was precipitated with ammonium acetate and divided into two equal aliquots. One aliquot of each sample was cut with 5 U *Nla*3 (New England Biolabs, Boston, MA) according to the conditions of the manufacturer in a final volume of 25 μL for 13 h at 37 C, and the second aliquot was incubated without enzyme. Eight microliters of each reaction mixture were analyzed on a 6% polyacrylamide gel, followed by staining with ethidium bromide.

Results

Pathological examination of the two fragments of rib revealed that one was normal (Fig. 2A), whereas the other demonstrated the changes of fibrous dysplasia (Fig. 2B).

DNA was obtained from bone of a normal control, from the subject's unaffected and affected rib, and from mononuclear leukocytes of the subject. After PCR amplification

TABLE 1. Primers for amplification and sequencing $G_s\alpha$ genomic DNA and cDNA

PCR primers		
GCGCTGTGAACACCCCACGTGTCT	5′-Primer for $G_s\alpha$ DNA amplification (located in the intron perceding exon 7)	PCR primer 1
CGCAGGGGGTGGGCGGTCACTCCA	3′-Primer for $G_s\alpha$ DNA amplification (located in the intron following exon 10)	PCR primer 2
GAATGTCAAGAAACCTTGATCTCTGTT	3′-Primer for amplifying $G_s\alpha$ DNA for restriction enzyme analysis	PCR primer 3
TGCTACGAACGCTCCAACGAGTACC	5′-Primer for $G_s\alpha$ cDNA amplification (nucleotides 484–508)	PCR primer 4
TAGCATCCTCAGGAGTAGTGTAGCG	3′-Primer for $G_s\alpha$ cDNA amplification (antisense to nucleotides 973–949)	PCR primer 5
Reverse transcriptase primer		
CATCTCGAATGAAGTACTTGGCCCGGGTCACGCGT	Reverse transcriptase primer for $G_s\alpha$ cDNA (antisense to nucleotides 1030–996)	Reverse transcription primer
Sequencing primers		
ACTACTCCAGACCTTTGC	Sequencing primer for $G_s\alpha$ Arg^{201} position in amplified DNA	Sequencing primer 1
CAGTAAGCCAACTGTTACC	Sequencing primer for $G_s\alpha$ Gln^{227} position in amplified DNA	Sequencing primer 2

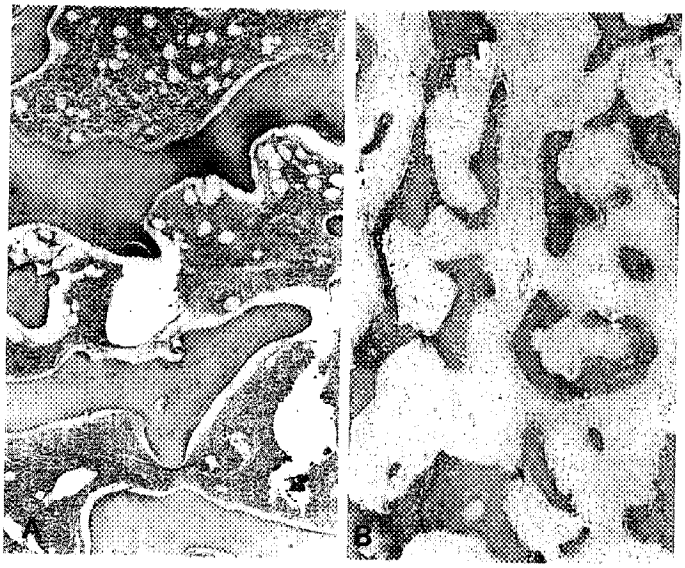

FIG. 2. Hematoxylin- and eosin-stained sections (original magnification, ×40) of bone from normal rib (A) and the affected area showing fibrous dysplasia (B). The affected area shows smaller, poorly oriented trabeculae, and the marrow cells have been replaced by tightly packed, spindle-shaped cells. Under polarized light and reticulin stain (not shown), the affected bone appeared woven rather than lamellar. The intertrabecular spaces consisted of benign cellular fibrous stroma.

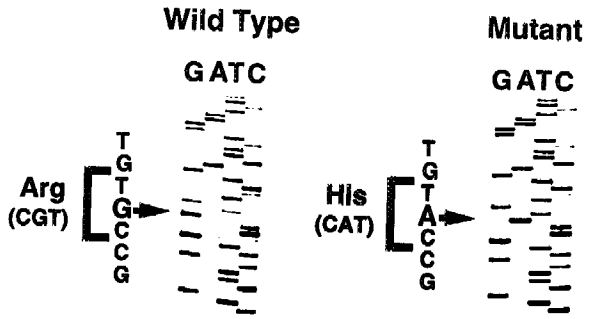

FIG. 3. Wild-type and mutant sequences of $G_s\alpha$. The regions of interest of $G_s\alpha$ were amplified from DNA extracted from bone of a normal control, from the subject's affected and unaffected ribs, and from mRNA extracted from the subject's affected rib. These fragments were subcloned and sequenced, as described in *Materials and Methods*. The frequency of the mutant sequence was 47% in DNA amplified from affected bone and 17% in DNA amplified from unaffected bone ($P < 0.05$). Only the wild-type sequence was found in the DNA extracted from bone of a normal control. The mutant sequence was found in 42% of the recombinant clones prepared by amplifying cDNA complimentary to mRNA of affected bone.

(PCR primers 1 and 2), a major band of 710 bp was identified, subcloned, and sequenced. Figure 3 shows examples of the two different sequences obtained for the codon of $G_s\alpha$ amino acid 201. The mutant DNA sequence predicts a His for Arg substitution. The A for G substitution at cDNA nucleotide position 602 was found in greater frequency in DNA from affected bone than in DNA from unaffected bone of the subject. Of the recombinant plasmids obtained from PCR-amplified DNA of the subject's affected and unaffected bone, the mutant sequence was found in 9 of 19 (47%) and 3 of 18 (17%), respectively ($P < 0.05$). Of the recombinant plasmids obtained from DNA of a normal control, 0 of 10 demonstrated the mutant sequence. To determine whether the mutant $G_s\alpha$ is expressed in affected bone, we analyzed RNA from affected bone. Six of 13 (42%) of the recombinant clones had the mutant sequence. Not shown is the normal codon for glutamine at position 227 seen in all recombinant plasmids from all samples.

The PCR-amplified $G_s\alpha$ DNA from the subject's MNL, affected bone, and unaffected bone was analyzed by restriction enzyme analysis. The 246-bp band created by the *Nla*3 restriction enzyme cut is darkest in the PCR-amplified DNA of affected bone and absent in the PCR-amplified DNA from mononuclear leukocytes (Fig. 4). This suggests that the frequency of the mutation is greatest in DNA from affected bone, intermediate in the DNA from unaffected bone, and least in the MNL DNA. This semiquantitative estimate is consistent with the relative frequencies of the individual recombinant clone sequences.

Discussion

We tested the hypothesis that this clinical variant of the MAS is caused by an activating mutation of $G_s\alpha$. The clinical presentation was unusual for the MAS. The subject presented with vaginal bleeding at age 3 yr, but without bone lesions. Although other patients with the incomplete form of MAS (sexual precocity and bone lesions without skin lesions) have been described, the presentation of sexual precocity alone is quite unusual (2, 16, 17). The subject developed normally through spontaneous puberty, suggesting that the sexual precocity was not sustained. A single lesion of fibrous dysplasia was discovered 24 yr after the first episode of menstrual bleeding, and no other characteristics of MAS were present. Usually, the lesions of fibrous dysplasia are an early finding in MAS, although they have been reported to develop 9 yr after other manifestations (3, 4). Therefore, this is an unusual presentation for MAS.

We have determined that a bone lesion of fibrous dysplasia

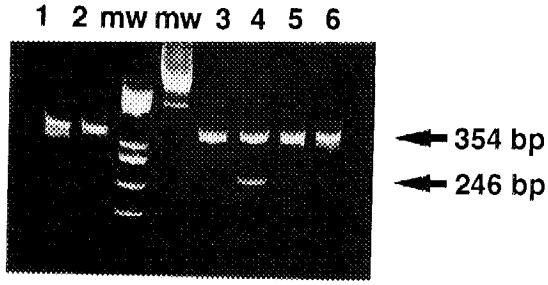

FIG. 4. Restriction enzyme analysis of amplified DNA from the subject's lymphocytes, affected bone, and unaffected bone. Lanes 1 and 2, Amplified MNL DNA. Lanes 3 and 4, Amplified DNA from affected bone. Lanes 5 and 6, Amplified DNA from unaffected bone. Lanes 2, 4, and 6, Amplified DNA cut with *Nla*3. Mol wt markers are in lanes marked mw. DNA (150 ng) from the indicated sources of the subject was amplified, as described in *Materials and Methods*, using PCR primers 1 and 3. The amplified DNA (354 bp) was precipitated with ammonium acetate, an aliquot was cut with *Nla*3, and the DNA was analyzed on a 6% polyacrylamide gel electrophoresis minigel with ethidium bromide staining, as described in *Materials and Methods*. Amplified DNA from MNL did not yield a visible 246-bp fragment when cut with *Nla*3 (lane 2). Amplified DNA from affected and unaffected bone was partially cleaved by *Nla*3 to yield a 246-bp fragment (lanes 4 and 6). The 246-bp fragment generated from amplified DNA of affected bone (lane 4) stained more intensely than that of unaffected bone (lane 6).

in this subject contains the His for Arg substitution at $G_s\alpha$ position 201. The His^{201} substitution has been reported in nonosseous affected tissues of other patients with MAS (6, 7). Therefore, a different mutation of the DNA cannot explain the unusual clinical presentation. It seems more likely that the mutation can occur at different stages of embryonic development, resulting in different degrees of mosaicism and, subsequently, different degrees of clinical involvement. The initial studies by Weinstein and colleagues (6) did not analyze DNA from bone. We were successful because we had access to freshly frozen bone. We found the normally occurring Gln at position 227. Mutations of Gln^{227} can also activate $G_s\alpha$. Such mutations have been proposed to cause endocrine overactivity in specific tissues (12, 18), but have not yet been observed in MAS (19). We also investigated whether the message for the mutant $G_s\alpha$ was expressed in the bone lesions. Our results suggest that it is expressed at a frequency similar to the frequency found in bone DNA. As the message for the mutant $G_s\alpha$ is present in bone, it seems likely that the mutant $G_s\alpha$ protein is also expressed in this tissue. The relatively high frequency of the mutant message in the osseous lesion from this subject is in contrast to the absence of mutant message in skin lesions from other subjects (7). The expression of the mutant message has not yet been reported in other tissues.

The frequency of the mutant DNA sequence was greater in affected bone than in unaffected bone. The greater frequency of the mutant sequence in affected bone may be the result of random distribution, and/or it may reflect proliferative advantage of the cells carrying the mutant sequence. Unaffected bone may be a source of DNA for establishing the diagnosis in other patients.

Although we have studied a single affected tissue from a single subject with MAS, these results are sufficiently compelling to help define the clinical characteristics of this syndrome. First, the same activating mutation of $G_s\alpha$ is associated with widely variant clinical syndromes. Second, the DNA mutation of the bone lesions is qualitatively identical to that found in other affected tissues of other patients with MAS (6). Third, the mutant $G_s\alpha$ is expressed in bone lesions. Fourth, a simple restriction enzyme assay can be applied to screen for the presence of the His^{201} substitution in affected and unaffected tissues. Fifth, unaffected bone is a source of mutant $G_s\alpha$ DNA and may be useful in establishing the diagnosis of MAS.

In summary, we have analyzed the sequence of $G_s\alpha$ at amino acid positions 201 and 227 in the affected and unaffected bone and lymphocytes of a subject with an unusual presentation of MAS. We conclude that the $G_s\alpha$ with the His^{201} substitution, which is probably responsible for the more classic presentations of the syndrome, is expressed and probably responsible for the unusual clinical manifestations in our subject.

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